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Redox Regulation of Enzyme Activity During Wood Decay

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ABSTRACT

A potential strategy in the search for alternative wood preservatives against fungal decay is to target the extracellular wood-decay process itself, rather than the decay organisms. This presents novel targets for selective disruption and possibly without the broad-spectrum toxicity associated with conventional wood preservatives. The enzymes of white rot decay are mechanistically diverse (e.g. hydrolytic, oxidative, peroxidative) and therefore various strategies for the disruption of their activity can be conceptualized. We have characterized how effecters control activity of the extracellular enzyme glyoxal oxidase. This enzyme is secreted by *Phanerochaete chrysoporium* and produces hydrogen peroxide required by ligninolytic peroxidases. Our studies with recombinant glyoxal oxidase show that the native enzyme is activated by inorganic oxidants or by lignin peroxidase when peroxidase substrates of high redox potential are used. The interconversion between active and inactive forms of the enzyme is defined in redox terms based on spectroelectrochemical measurements of the active site of glyoxal oxidase.

Keywords: enzyme inactivation, white rot, peroxide

INTRODUCTION

Increasing concerns over the environmental impact of conventional wood preservatives have provided impetus to look for alternatives. A prime reason for the environmental concern is the broad-spectrum toxicity of the preservatives i.e. they are toxic to many organisms, not just the wood-decay organisms. A potential strategy in the search for alternative preservatives against fungal decay is to target the extracellular wood-decay process itself, rather than the decay organisms. Knowledge of the enzymes, non-enzymic catalysts, metabolic pathways, and regulatory mechanisms is necessary for a rationale approach (Kersten, 1994). Here we present evidence for regulatory mechanisms used by the white rot fungus *Phanerochaete chrysosporium* for the control of enzyme activity related to wood decay.

Extracellular enzymes of the lignin-degrading system of *P. chysosporium* include lignin peroxidase (LiP), manganese peroxidase (MnP) and the H_2O_2-producing enzyme glyoxal oxidase (GLOX). Peroxide generated by GLOX is coupled to the peroxidase reactions for

the oxidations of Mn2+ and aromatic substrates. Specifically addressed here are the interactions between GLOX and LiP that regulate peroxide generation and consumption. For a general review of enzymes of lignocellulose degradation see Cullen and Kersten (1992).

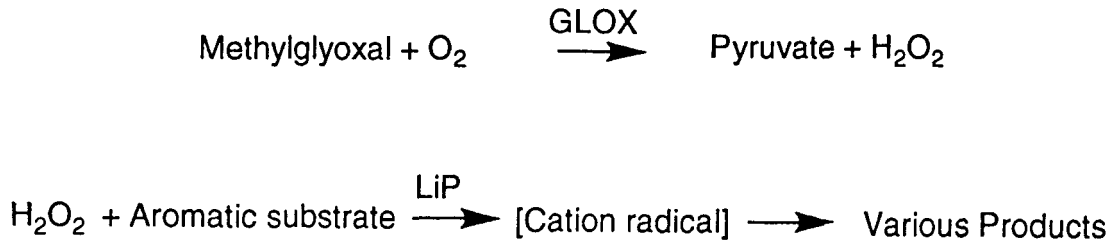

Figure 1. Glyoxal oxidase and lignin peroxidase of *P. chysosporium.* The peroxide generated by GLOX is coupled to the oxidations catalyzed by LiP. Reactive oxidized intermediates are generated by LiP and undergo further spontaneous reactions.

LiP was discovered based on the H_2O_2-dependent C_α - C_β cleavage of lignin model compounds and subsequently shown to catalyze the partial depolymerization of methylated lignin *in vitro* (Glenn *et al.,* 1983; Gold *et al.,* 1984; Tien *et al,* 1983; Tien *et al.,* 1984). There are several isozymes of lignin peroxidase, all of which are glycoproteins of molecular weights estimated at 38-46 kDa (Kirk *et al.,* 1986; Leisola *et al.,* 1987; Renganathan *et al,* 1985). The underlying principle behind the array of reactions catalyzed by LiP is explained by the ability of the peroxidase to oxidize the aromatic nuclei of substrates by one electron; the resulting aryl cation radicals degrade spontaneously via many reactions dependent on the structure of the substrate and on the presence of reactants (figure 1).

GLOX is a physiological source for the peroxide required by LiP for its oxidations. This oxidase can use simple aldehyde and α -hydroxycarbonyl compounds for the reduction of molecular oxygen to H_2O_2 (Kersten, 1990). The metabolic pathway for the production of GLOX substrates is not fully understood but two substrates, methylglyoxal and glyoxal, are found in ligninolytic cultures grown on glucose. Another source of GLOX substrates is lignin itself; the fragmentation of the arylglycerol β -aryl-ether substructure produces glycolaldehyde that perpetuates the oxidation with GLOX (Hammel *et al.,* 1994). The gene encoding GLOX has been fully sequenced, the genetic organization determined, and the recombinant protein (rGLOX) produced and secreted with *Aspergillus* (Kersten and Cullen, 1993; Kersten *et al,* 1995).

The heterologous expression of GLOX has provided the technical capability to study in detail the reversible activation of the oxidase that was first observed with purified enzyme from *Phanerochaete* (Kersten, 1990). The oxidase is inactive unless coupled to an effective peroxidase reaction. The requirements in this coupled peroxidase reaction to activate rGLOX has been studied with respect to the type of peroxidase and the

peroxidase substrate (Kurek and Kersten 1995). Furthermore, the redox nature of the interconversion between inactive and active GLOX has been defined (Whittaker *et al.,* 1996).

REGULATION OF GLOX ACTIVITY BY LiP ACTIVITY

We have characterized the activation and inactivation of rGLOX with three classes of peroxidase substrates: non-phenolic monomers, phenolic monomers, and polymeric lignin (Kurek *et al.,* 1995). Non-phenolic methoxybenzenes were chosen because the homologous series of methoxybenzenes vary in redox potential and have been useful in characterizing the oxidations catalyzed by LiP, horseradish peroxidase, laccase (Kersten *et al.,* 1990) and MnP (Popp *et al.,* 1991). All tetramethoxybenzenes (TMBs) used in this study are readily oxidized by LiP, whereas anisole is not (Kersten *et al.,* 1990), Guaiacol is slowly oxidized by LiP and H_2O_2 at pH 4.5.

The first report of the activation of GLOX with a peroxidase was described with LiP and the secondary metabolize veratryl alcohol. Veratryl alcohol is not unique in the activation of rGLOX by a LiP-coupled system; 1,2,4,5-TMB ($E_{1/2}$ = O.81V versus a saturated calomel electrode), 1,2,3,5-TMB ($E_{1/2}$ = 1.09V) and 1,2,3,4-TMB ($E_{1/2}$ = 1.25V) also activate the oxidase very effectively (figure 2). Even though guaiacol was oxidized in the coupled system (as evidenced by spectrophotometric measurements), activation of rGLOX was marginal. Furthermore, guaiacol has no effect on the progressive inactivation of rGLOX in the absence of a peroxidase, but strongly inhibits the oxidase activation with veratryl alcohol and LiP. The response with catechol was unusual in that it inactivated rGLOX even prior to LiP addition, suggesting a different mode of action. Moderate activation of rGLOX was also observed with macromolecular spruce milled wood lignin plus LiP.

An explanation for these results is that there is efficient activation when the coupled peroxidase generates oxidizing species (e.g. cation radicals) from its substrates; these oxidizers then activate GLOX. Indeed, the best nonphenolic substrates for the peroxidases provide the best activation. Also, poor peroxidase substrates yield moderate activation of the oxidase probably due to the low amounts of rGLOX activating intermediates formed. A direct consequence of this weak peroxidase reaction is the accumulation of H_2O_2. The inactivation with catechol may then be explained by its direct reduction of GLOX. The redox nature of this interconversion of active and inactive forms of rGOX was confirmed by spectroelectrochemical measurements with inorganic oxidants.

ACTIVATION OF GLOX BY INORGANIC OXIDANTS.

In addition to the activation of GLOX by lignin peroxidase coupled reactions (above), the oxidase can also be oxidatively activated with high potential inorganic oxidants including molybdicyanate ($K_3Mo(CN)_8$), hexachloroiridate (Na_2IrCl_6) and Mn^{3+} EDTA (Whittaker *et al.,* 1996). Dialysis of the activated enzyme results in reversion of the enzyme back to the inactive form. The oxidized and reduced forms of the ezyme show distinct spectral

characteristics and thus allow measurement of the relative proportion of enzyme forms in solution. Spectroelectrochemical measurements provide an estimate of the redox potential ($E_{1/2}$ = 0.64V versus NHE) governing this interconversion of enzyme states and that it is a one-electron transition (figure **3).**

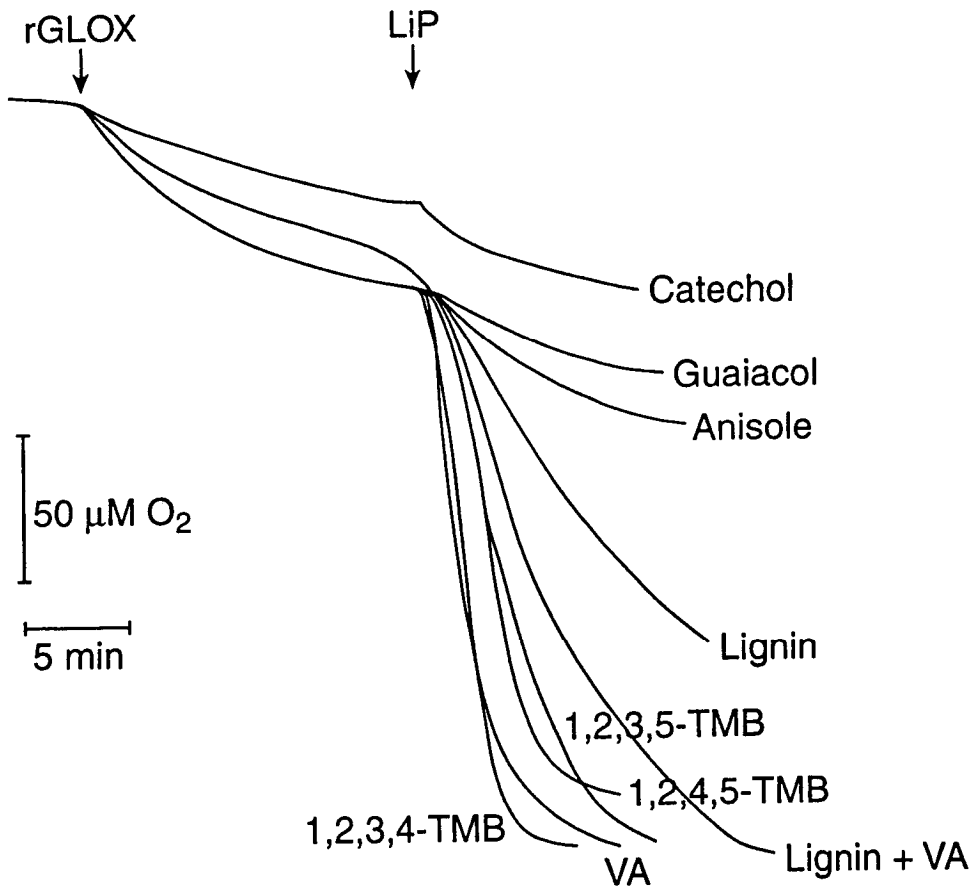

Figure 2. Activation of rGLOX with LiP substrates. Reaction conditions were with 4.5 nkat/ml rGLOX and peroxidase substrate as indicated at pH 4.5. After inactivation of oxidase, LiP was added. When lignin was used as the peroxidase substrate, 5 % dimethylformamide was also included in the reaction medium. VA: veratryl alcohol. Figure from Kurek and Kersten, 1995.

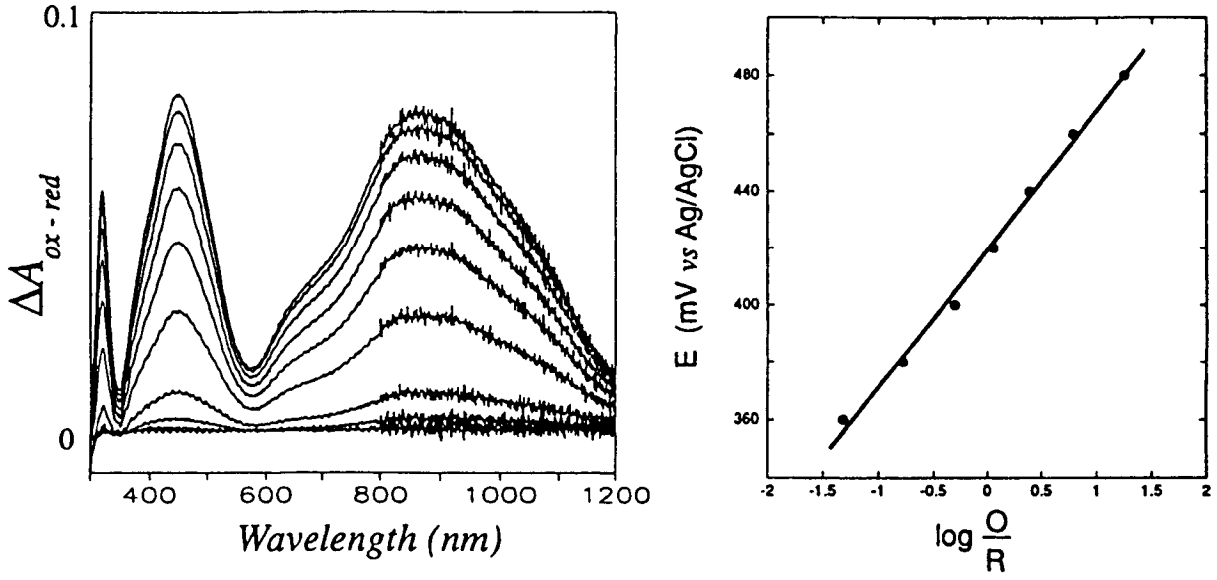

Figure 3. Spectroelectrochemical oxidation of glyoxal oxidase. Left panel: native GLOX (2.2 mM) in 10 mM sodium phosphate, pH 7, containing 2 mM $K_4Mo(CN)_8$ was progressively oxidized under applied potentials ranging from 0.34 to 0.5 V (versus Ag/AgCl) in 20 mV increments, Right panel: Nernst plot of potential/absorption data for redox conversion of GLOX from reduced (inactive) to oxidized (active) states. The theoretical line determines that $E_{1/2}$ = 0.64 versus NHE and n = 1.2. Figure from Whittaker *et al.*, 1996.

CONCLUDING REMARKS

To effectively and specifically disrupt the metabolism of wood decay by fungi, an essential component of the decay process must be targeted. GLOX appears to have a physiological role in 1) supplying peroxide for ligninolytic peroxidases, 2) oxidizing carbohydrate fragments and thus supplying an assortment of metabolizes of yet undetermined significance, and, 3) regulating the supply of peroxide by interactions with a redox sensitive switch at the active site of GLOX. Rigorous proof for an essential role in lignin metabolism is difficult and generalizations to fungal systems other than *P. chrysosporium* are premature. However, the described system provides a useful context for the conceptualization of how potential wood preservatives, as well as those in present use, could have modes of action.

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